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A Comprehensive Review on Molecular Characteristics and Food-Borne Outbreaks of *Listeria monocytogenes*

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Abstract—*Listeria monocytogenes* is an important foodborne pathogen which causes serious invasive illness, and affects mostly elderly and immune-compromised people, pregnant women, newborns and infants leading to listeriosis. *L. monocytogenes* can cause vast outbreaks due to consumption of contaminated food products, and has a significant role in public health. The pathogen has been isolated from food, human and animal samples world-wide. Neonatal listeriosis is most commonly reported incase of humans, where as in animal populations, spontaneous abortions, meningoencephalitis and endometritis are the most common. The purpose of this review is to enumerate *Listeria* epidemiology world-wide by using publicly available data from CDC, FDA and ProMED and by describing the details such as countries involved, source, suspected and confirmed case counts etc. to understand its public health importance. This review also offers a description of bacteriological characteristics, taxonomy, virulence determinants, typing methods, a detailed account of listeriosis in human and in animals and an up-to-date information of the recent outbreaks of *L. monocytogenes.* We specifically aimed at the prevalence and epidemiology of *L. monocytogenes* globally, since it is a major food-borne pathogen and is the third leading cause of death due to food poisoning. This review paper provides information on *L. monocytogenes* to understand the better management of the infection, the source of infection and route of transmission of the disease. Most of the listeriosis cases were linked with the consumption of contaminated food and it is important to identify the type of food materials to mitigate the risk of Listeriosis in the high-risk populations.

Keywords: *Listeria monocytogenes*, *Listeriosis, Foods, Epidemiology, Outbreak, Prevalence*

INTRODUCTION

Listeria monocytogenes is a foodborne pathogen that causes severe invasive illness, mainly in elderly and immuno-compromised persons, pregnant women and infants. Listeriosis, a disease caused by an opportunistic food-borne pathogen *Listeria monocytogenes* causes invasive syndromes with more than 30% fatality rate. Listeriosis occurs in animals and humans mainly from the consumption of contaminated food (Barbuddhe *et al*. 2012). The genus *Listeria* is closely related to the genus *Bacillus*, *Enterococcus*, *Streptococcus*, *Staphylococcus* and *Clostridium*. Being facultatively anaerobic, the *Listeria* spp. are 0.4 by 1-1.5 mm in size, does not form spores, have no capsule and show motility at 10-25°C (Rocourt 1999).

Listeria monocytogenes was classified in the family *Corynebacteriaceae* in Bergey's Manual of Determinative Bacteriology (Stuart and Pease 1972), but later it was listed in a section named Regular, Nonsporing Gram-Positive Rods together with *Lactobacillus*, *Erysipelothrix* and *Brochothrix* (Seeliger and Jones 1986). Till 1961, it was the only recognized species within the genus *Listeria*, and *L. denitrificans*, *L. grayi* and *L. murrayi* were added to the genus between 1961 to 1971 (Rocourt *et al*. 1982). The non-pathogenic strains of *L. monocytogenes* belonging to serovar 6 were separated into a new species, *L. innocua* in

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1983 and later *L. welshimeri* and *L. seeligeri* were also added (Seeliger and Jones 1986). The species *L. denitrificans* is quite different from other *Listeria* (Jones 1988) and it was re-classified based on the numerical taxonomy, DNA base composition and DNA-DNA hybridization (Stuart and Weishimer 1974). The taxonomic position of the genus *Listeria* now includes the species *L. monocytogene*s, *L. innocua*, *L. ivanovii*, *L. seeligeri*, *L. grayi*, *L. welshimeri* and *L. murrayi* in one group and the other in *L. denitrificans* based on numerical taxonomy as well as DNA homology and 16S rRNA cataloging (Rocourt 1988).

The ISO Standard 11290, part 2 (ISO 1998) and optional protocols introduced by FDA and USDA methods are used for enumeration of *L. monocytogenes*. Samples are incubated in enrichment broths supplemented with antibiotics, such as Buffered Listeria Enrichment Broth, Half-Fraser Broth and Fraser Broth for all the methods. The cultures are streaked on selective agars such as PALCAM Agar where *Listeria* spp. show small brown/black colonies with black halos, Listeria Selective Agar (Oxford) with the same characteristic as in PALCAM and Chromogenic Listeria Agar (ALOA) where *Listeria* spp. show blue/ green colonies (Jeyaletchumi *et al*. 2010). The bacterial colonies observed under reflected light are smooth, slightly flattened and milky white. The colonies show a characteristic blue/green colour illuminated by obliquely transmitted light, a technique called Henry's lamp technique. *Listeria monocytogenes* also shows CAMP reaction: the ability to haemolyse in horse or sheep red blood cells cultured with *Stapphylococcus aureus*. It is catalase positive and oxidase negative (Low and Donachie 1997).

The first human Listeriosis case was reported in 1929 in Denmark (Nyfelt 1929) and *L. monocytogenes* was first cultured from a patient with meningitis (Seeliger 1988). Reports on isolation of *Listeria* was persistent between 1970s and 1980s, and a sequence of epidemic outbreaks started in humans in North America and Europe, and listeriosis was clearly recognized as an important food-borne infection (Bille 1990) from 1983 onwards. The foods mainly involved are the industrially produced, refrigerated ready-to-eat products that are eaten without cooking or reheating such as soft cheese, dairy products, pates, sausages, smoked fish and salads (Rocourt 1996). In case of ruminants, Listeriosis can be transmitted by consumption of spoiled silage where the bacteria multiply readily, resulting in flock outbreaks (Vazquez *et al*. 1992). Both outbreaks and sporadic cases of listeriosis in European Union (EU) were reported in 2013 with 1763 confirmed human cases in 27 member states (EFSA 2013) -an increase by 8.6% compared to that in 2012. In all, 99.1% of the cases were hospitalized and listeriosis was recorded as the most prevalent among all zoonoses under EU surveillance (Robert *et al*. 2017). The large scale outbreak that occurred in South Africa during 2017 - 2018 brought to light the potential of *Listeria* to cause widespread disease. This outbreak is the largest till now with more than 1000 laboratory confirmed cases (Angel *et al*. 2019).

This review has been prepared by using publicly available data to report trends in *Listeria* epidemiology through an analysis of CDC, FDA and ProMED reports for better understanding the public health importance of *L. monocytogenes*. This review addresses detailed information relating to bacteriological characteristics of the organism with its virulence determinants, the listeriosis disease in human and animals, and also provides the up to date information of recent outbreaks till date from food materials associated with *L. monocytogenes.*

MOLECULAR CHARACTERISTICS AND STRAIN TYPING

Being an intracellular pathogen, *L. monocytogenes* has evolved over a long period of time with unique properties and functions. It enters into the host by ingestion of contaminated food and can resist to the host proteolytic enzymes in the acidic stomach environment, non-specific inflammatory attacks and bile salts with the help of many stress-response genes (*opu*CA, *lmo*1421 and *bsh*) and the related proteins (Sleator *et al*. 2003). With the help of a family of surface proteins called internalins (InlA and InlB), it adheres and enters into host cells (Gaillard *et al*. 1991). With the 88 kDa protein encoded by *inlA* and the 65 kDa protein encoded by *inlB*, *L. monocytogenes* recognizes E-cadherin and C1q-R (or Met) receptors on cell surface to enter easily into many host-cell types, such as hepatocytes, fibroblasts and epithelioid cells, and elude host immune monitoring functions (Vazquez-Boland *et al*. 2001). Primarily *L. monocytogenes* is located in single-membraned vacuoles following its uptake by host cells. Listeriolysin O (LLO) and phosphatidylinositol-phospholipase C (PI-PLC) are the two virulence associated molecules that are responsible for lysis of the primary single-membraned vacuoles and successive escape by *L. monocytogenes*. LLO is a pore-forming, thiol-activated toxin; a 58 kDa protein (encoded by *hlyA*) essential for *L. monocytogenes* virulence (Portnoy *et al*. 1992). PI-PLC, a 33 kDa protein encoded by *plcA*, interacting with a 29 kDa protein encoded by *plcB*, the phosphatidylcholine-

phospholipase C (PCPLC), assist LLO in the lysis of the primary vacuoles (Vazquez-Boland *et al*. 2001). *Listeria monocytogenes* is released into the cytosol and then undergoes intracellular growth and multiplication after lysis of the primary single-membraned vacuoles. ActA, a 67 kDa surface protein aids in the intracellular adaptability and cell-to-cell spread of *L. monocytogenes*, co-transcribed with PC-PLC and helps in the formation of polarized actin tails to propel it toward the cytoplasmic membrane. The bacteria become enveloped in the membrane with filopodium-like structures engulfed by the adjacent cells which result in the formation of secondary double-membraned vacuoles. The starting of a new infection cycle depends on PC-PLC activated by Mpl, a 60 kDa metalloprotease (Vazquez-Boland *et al*. 2001). The virulence-associated proteins LLO, Mpl, ActA, PIPLC and PC-PLC are placed in a 9.6 kb virulence gene cluster (Gouin *et al*. 1994) regulated by a 27 kDa protein (PrfA) encoded by *prf*A (pleiotropic virulence regulator). The *prfA* gene, co-transcribed with the *plcA* gene, activates the transcription of *L. monocytogenes* virulence-associated genes. In addition to these, an invasion-associated protein, *Iap* is also associated with *L. monocytogenes* virulence and pathogenicity (Vazquez-Boland *et al*. 2001).

Based on the results of different genotyping methods, *L. monocytogenes* was divided into three genetic lineages (Wiedmann *et al*. 1997a). Serotypes 1/2b, 3b, 4b, 4d and 4e were added into Lineage I (Cheng *et al*. 2008). Among strains of different serotypes, 4d and 4e strains are scarce among clinical and food samples and have similarity with 4b strains (Cheng *et al*. 2008). Therefore, serotypes 4b, 4d and 4e describes the subcluster in Lineage I known as ‘‘serotype 4b complex” (Eifert *et al*. 2005). Serotypes 1/2a, 1/2c, 3a and 3c were added in Lineage II and serotypes 4a, 4c and some strains belonging to serotype 4b were added in Lineage III (Cheng *et al*. 2008). Lineage III isolates were divided into three distinct subgroups, IIIA, IIIB, IIIC (Liu *et al*. 2006). A fourth phylogenetic lineage was also added later as Lineage IIIB, reclassified as Lineage IV which belongs to a distinct group and also shows phylogenetic differences from other lineages (Orsi *et al*. 2011). The Lineages I and II have been mainly found in sporadic and outbreak cases of human listeriosis (Cheng *et al*. 2008). Lineage II and some Lineage I strains (serotypes 1/2b and 4b) are widely distributed in natural and farm environments, and also found in foods (Kathariou 2002). Lineages III and IV have shown significant biodiversity but are rare (Ward *et al*. 2010), associated with non-primate mammals and ruminants (Cheng *et al*. 2008).

Since *L. monocytogenes* contains numerous strains, it is important to have a vigorous system of subtyping to track individual strains, to examine the epidemiology and population genetics, and to control outbreaks associated with Listeriosis. The subtyping methods also help to track the source of contamination in food processing environments which is essential to develop control strategies. Since the phenotypic subtyping methods have low differentiation ability, the genotypic methods are more reliable.

Although plasmid typing was used in combination with DNA fingerprinting as a typing method to confirm *L. monocytogenes*, it seems that it has not much value as a typing tool, because most strains of *L. monocytogenes* do not contain plasmids (Farber and Peterkin 1991).

Pulsed-Field Gel Electrophoresis (PFGE)

PFGE uses restriction enzymes which cut the genomic DNA resulting in many different fragments ranging between 40 and 600 kb. PFGE has been considered as the gold standard tool to study epidemiology in foodborne outbreaks. It is a sensitive method which tracks genetic changes like point mutations, deletions, insertions and transpositions (Jadhav *et al*. 2012). A study was carried out in China by Luo *et al.* (2017) to investigate the prevalence of *L. monocytogenes* in raw pork from open markets where they performed PFGE by using *AscI* restriction enzyme and MLST. They found Simpson’s index of diversity as 0.8793 and 0.7842 for PFGE and MLST, respectively. The 262 *L. monocytogenes* isolates generated 39 different pulsotypes in PFGE in their study. Oliveira *et al.* (2018) from Brazil isolated *L. monocytogenes* from chicken samples at industrial slaughterhouse and studied genetic relationship among the isolates by PFGE using macro-restriction profile with *ApaI,* which revealed 12 different pulsotypes. They found high diversity in pulsotypes among the isolates from carcass and drumettes. Nadia *et al.* (2018) screened *L. monocytogenes* in 1.5% food samples from Tetouan, North-Western of Morocco. The isolates belonged to serogroups 4b and 2a, and revealed eight different pulsotypes in (*AscI/ApaI*) combined PFGE profiling.

Multi-Locus Sequence Typing (MLST)

MLST based on DNA sequencing is used for genetic subtyping of *L. monocytogenes* which targets multiple genes or gene fragments to determine the subtypes and

the genetic relatedness among isolates. MLST is less ambiguous and easy to interpret compared to PFGE. MLST plays an important role in subtyping of *L. monocytogenes* and in phylogenetic studies due to the lower cost of DNA sequencing and the results can be exchanged easily between different laboratories giving reliable and unambiguous data (Jeyaletchumi *et al.* 2010). A study was carried out by Abbey *et al.* (2018) in the Upper Great Plains states of US to evaluate the genetic diversity of clinical listeriosis isolates from ruminants using MLST.

Repetitive Extragenic Palindrome-Polymerase Chain Reaction (REP-PCR)

In REP- PCR, known conserved regions can be amplified by using only one DNA primer which gives polymorphic DNA fingerprints. *Listeria monocytogenes* contains a genome which has randomly disseminated repetitive sequence elements, such as repetitive extragenic palindromes (REPs) of 35–40 bp length, an inverted repeat, and enterobacterial repetitive intergenic consensus sequences (ERICs) with 124–147 bp length, a highly conserved central inverted repeat. In order to discriminate species and strains, REP and ERIC sequences exhibit functional primer binding sites for PCR amplification in the *L. monocytogenes* genome. REP-PCR gives similar level of discrimination with PFGE; hence it can be used as an alternative method for the fast-track subtyping of *L. monocytogenes* (Jeyaletchumi *et al.* 2010). Hadjilouka *et al.* (2014) carried out REP-PCR for 121 *L. monocytogenes* strains isolated from food products and found better differentiation among isolates. REP-PCR possesses a discriminatory power similar to PFGE and ribotyping. It is also faster and cheaper than other typing techniques. Hence, it is considered as an important typing method for *L. monocytogenes*. Soni *et al.* (2015) carried out a study to characterize *L. monocytogenes* isolated from pregnant women using ERIC- and REP-PCR methods. Both these methods collectively showed that the isolates from placental bit and vaginal swab had distinct DNA fingerprints except a few isolates with identical patterns.

LISTERIOSIS IN HUMANS

With the help of a variety of virulence factors, *L. monocytogenes* can invade the host and survive within the host cells. It can colonize the human hosts using phagocytic cells. After adhesion and invasion of the intestinal epithelium, it proliferates throughout the body and infects macrophages, epithelial cells, endothelial cells, hepatocytes, fibroblasts and neurons. With the help of the surface protein internalin A (encoded by *InlA* gene), it translocates from the lumen into the intestinal epithelial cells. InlA protein binds to glycoprotein E-cadherin present on the host cell, which helps in the entry of *L. monocytogenes* into the cell. After internalization, a pore-forming cytolysin listeriolysin O (LLO) encoded by *hlyA* gene and two phospholipases C encoded by the genes *plcA* and *plcB*, help the pathogen to escape from phagosomes. With the help of an actin polymerizing protein ActA, it spreads to the neighbouring cells. *Listeria monocytogenes* can proliferate by the bloodstream to mesenteric lymph nodes, liver, spleen and multiply within host cells. The liver and spleen are the first organs affected. These organs release the bacterium into the blood stream resulting in septicaemia. The organism can cross the placental barrier which leads to abortion or generalized neonatal infections such as pneumonia, sepsis and meningitis, and can also cross the blood–brain barrier causing meningitis, meningoencephalitis and rhombencephalitis (Anderson *et al.* 2016).

Different serotypes of *L. monocytogenes* have been isolated from foods, while serotypes 1/2a, 1/2b and 4b are responsible for majority of clinical cases worldwide (Kathariou 2002). Many listeriosis outbreaks in the past have been associated with closely related clones of serotypes 1/2a and 4b (Lomonaco *et al*. 2013). The annual incidence of listeriosis ranges between 0.1 and 11.3 cases per million population worldwide (Swaminathan and Gerner 2007). During 1980–2000, serotype 4b strains were responsible for several outbreaks. In Europe and North America, serotype 1/2a was more frequently associated with the outbreaks of listeriosis (Cartwright *et al*. 2013). The rate of incidence was higher during the 1980s, but the number of human listeriosis cases reported was lower during the 1990s in Europe. The cases of listeriosis have increased since 2000 in the European Union (EFSA 2007). In France from 2005 to 2007, 46% increase in listeriosis incidence was observed, mainly in people of >60 years of age. Similar cases were also reported in France (Goulet *et al*. 2008).

Listeriosis predominantly affects immuno-compromised individuals of older age group (EFSA and ECDC 2014). It can result either in a non-invasive gastrointestinal form or an invasive clinical syndromes (Swaminathan and Gerner 2007). Risk of listeriosis has been found to increase among women of reproductive age and pregnant women (Pouillot *et al*. 2012). Materno-fetal listeriosis usually leads to abortion at a rate inversely proportional to the month of gestation (Awofisayo *et al*. 2015).

Sporadic and epidemic forms of listeriosis have been reported throughout the world. In India, the epidemiological data available on listeriosis is not sufficient to evaluate the extent of infection in human and animals. Due to the lack of reliable and rapid diagnostic test and also lack of required notification of listeriosis, the disease remains undiagnosed and under-reported (Barbuddhe *et al*. 2004). About one-third of human listeriosis cases in USA were reported during pregnancy resulting in spontaneous abortion in second or third trimester (CDC 2005). In England and Wales, 10–20% of cases were associated with pregnancy and neonatal disease that lead to abortion and stillbirth with 15–25% of infection (McLauchlin *et al*. 2004). In India, genital listeriosis has been reported most commonly. *Listeria monocytogenes* was found to be one of the causes of abortion and premature births (Bhujwala *et al*. 1973). There was no report of isolation of *L. monocytogenes* from the cervix of unsuccessful pregnancy, and of unhealthy cervical and vaginal discharges (Dhawan and Dhall 1963). However, *L. monocytogenes* was isolated 14% of 150 patients from the cervix with past history of abortions and miscarriages (Krishna *et al*. 1966), 1.34% of women with a bad obstetric history (Bhujwala and Hingorani 1975) and 10% of women with a record of abortions (Stephen *et al*. 1978). Moreover, 3.3% cases of spontaneous abortions were due to *L. monocytogenes* with a history of abortion in earlier pregnancy (Kaur *et al*. 2007), and cases of meningitis and hydrocephalus in children born from infected mothers was also reported (Gogate and Deodhar 1981). *Listeria monocytogenes* was isolated from the blood sample taken from a 4 hours old newborn, one and a half month old child having congenital heart disease and digestive failure, and a 1.5 year old child severely ill-fed (Gupta *et al*. 1997). The organism was also isolated from a 5 year old malnourished child with Perinephric abscess having abdominal pain (Gomber *et al*. 1998). It was detected in one out of 43 samples of cerebrospinal fluid collected from patients (Pandit *et al*. 2005). Listeriosis case was reported as late onset type with characteristics such as hepatosplenomegaly, lymphadenopathy, cutaneous haemorrhages and meningitis (Raghuraman and Rupnarayan 1988). A meningitis case was reportedly caused by *L. monocytogenes* in a 17 year old immuno-compromised patient, in which it was isolated from the cerebropinal fluid, and the patient recovered after treatment with ampicillin (Kalyani *et al*. 2006). Perinatal listerial infection including abortion, stillbirth, neonatal sepsis and meningitis are the common clinical syndrome caused by *L. monocytogenes*. In neonatal listeriosis, symptoms develop within 7 days or classically within 1 or 2 days (Klein 2001). Previously, a number of cases of spontaneous bacterial peritonitis in cirrhosis caused by *L. monocytogenes* were reported worldwide. In India, a greater number of patients have advanced renal insufficiency, who need extent use of temporary dialysis catheters in Catheter related bacteraemia (Nirni *et al*. 2002). The incidence of listeriosis cases varies in different countries between 0.1 and 11.3/1,000,000 (Anon 2002) but no such information are available in India because of negligence of active surveillance system for human listeriosis. Such surveillance system is necessary for proper estimation of the disease. To keep lowering the morbidity and mortality as much as possible, the government should take steps for educating the consumers, especially the high risk groups.

PREVALENCE AND OUTBREAKS OF *LISTERIA*

Listeria monocytogenes is listed as the third leading cause of death due to food poisoning (den Bakker *et al*., 2010). At least, 90% of listeriosis cases are linked to the ingestion of contaminated food products (Liu *et al*. 2015). The prevalence of *L. monocytogenes* is summarised in Table 1.

In India, isolation of *L. monocytogenes* was reported from the meat samples of 6.6 to 7.0% goats (Rekha *et al*. 2006), 7.4% sheep (Barbuddhe *et al*. 2000), 3 to 6 % buffalo (Barbuddhe *et al*. 2002), and 8.l% poultry meat samples (Barbuddhe *et al*. 2003). The organism was recovered from fish and fishery products worldwide and the prevalence rate was reported to be lower in tropical fish (Karunasagar and Karunasagar 2000). *Listeria monocytogenes* was involved in numerous outbreaks of listeriosis associated with the consumption of milk and milk products (Lyytikainen *et al*. 2000). Isolation of pathogenic *L. monocytogenes* was reported from goat milk (1.56%) (Barbuddhe *et al*. 2000) and buffalo milk (6.25%) (Barbuddhe *et al*. 2002) samples.

Soni *et al*. (2013) isolated *L. monocytogenes* from Ganges water (8%), human clinical samples (1.7%) including placental bit (5.3%), and vaginal swab (1.3%), and cow milk (5.8%) in Varanasi, India. However, *L. monocytogenes* could not be recovered from pasteurized milk and milk products (cheese, butter and ice-cream) in their study. Biswas *et al*. (2018) reported isolation of *L. monocytogenes* from cattle faeces, raw milk and lassi, and dahi and ice-cream samples, respectively. Prevalence of *Listeria* spp. in chevon, mutton and swab samples was reported to be 1.82%, 3.21% and 6.66%, respectively (Alka *et al*. 2019). Pegu *et al.* (2017) reported prevalence of *L. monocytogenes* from 2.31% of

five species of freshwater fish from the North-eastern region of India and reported isolation of *L. monocytogenes* from intestine (1.03%), fish gill (0.85%) and flesh (0.43%) samples. Biswas *et al*. (2018) collected a total of 200 samples from West Tripura district, Tripura including 50 each of cattle faeces and raw milk and 100 samples of milk products. The overall occurrence of *L. monocytogenes* was 8.50% including in cattle faeces (6.0%), raw milk (8.0%), lassi (12.0%), dahi (16.0%) and ice-cream (12.0 %) samples.

Although in US, regulatory initiatives and industry actions were executed to reduce outbreaks during 1998 to 2008, listeriosis outbreaks from dairy products were still reported (Cartwright *et al*. 2013). A number of listeriosis outbreaks from celery, lettuce, cantaloupe, sprouts, stone fruit, ice cream and caramel apples were reported in the U.S since 2010. As indicated by European Food Safety Authority and European Center for Disease Prevention and Control, frequencies of *L. monocytogenes* contamination was found highest in ready to eat food products, meat and fish products in the period 2004–2006 (EFSA and ECDC 2014). A EU-wide baseline survey was performed to estimate the prevalence of *L. monocytogenes* in certain RTE foods (fish, meat and cheese) in 2010–2011 (EFSA 2013). In a study carried out in UK, human listeriosis was attributed mainly to RTE foods such as sandwiches, mixed salads, followed by fish and beef (Little *et al*. 2010). European Union listeriosis outbreaks corresponded to the consumption of contaminated cheese, acid curd cheese in 2006–2007, quargel cheese in 2009–2010, hard cheese made with pasteurized milk in 2011 and a fresh cheese in 2012 in Germany, Austria, Germany, Belgium and Spain, respectively (Yde *et al*. 2012). Around 50% of the outbreaks reported in the US have also been linked to cheese during 2009-2011 indicated in the Foodborne Outbreak Online Database (FOOD) from CDC (Lomonaco *et al*. 2015). A multistate outbreak took place by ricotta salata cheese produced in Southern Italy in 2012 spread over 14 States in the US with a total of 23 cases and five deaths (Lomonaco *et al*. 2015) leading a worldwide recall since no single case was reported outside the US with the same cheese which was sold in Canada, Egypt, Europe, Australia, Japan and Mexico. Many outbreaks have also been linked with community food service where mainly elderly or people with underlying conditions were infected in hospitals (Gaul *et al*. 2013) and home-delivered meal programs (Smith *et al*. 2011).

Table 1: Prevalence of *Listeria monocytogenes* Strain from Different Sources from India

Source of Sample	Prevalence (%)	References
Fish and fishery products	50	Karunasagar and Karunasagar (2000)
Goat milk	1.56	Barbuddhe *et al*. (2000)
Sheep meat	7.4	
Buffalo meat	3 to 6	Barbuddhe *et al*. (2002)
Buffalo milk	6.25	
Poultry meat	8.l	Barbuddhe *et al*. (2003)
Goat meat	6.6 to 7.0	Rekha *et al*. (2006)
Ganges water	8	Soni *et al*. (2013)
Placental bit	5.3	
Vaginal swab	1.3	
Cow milk	5.8	
Fish intestine	1.03	Pegu *et al*. (2017)
Fish gill	0.85	
Flesh fish	0.43	
Cattle faeces	6.00	Biswas *et al*. (2018)
Raw milk	8.00	
Lassi	12.00	
Dahi	16.00	
Ice-cream	12.00	
Chevon	1.82	Alka *et al*. (2019)
Mutton	3.21	

As of 2016, data collected on *L. monocytogenes* infections from European countries reported 2555 listeriosis cases in the European Union, highest (1.3 per 100000 population) number of confirmed cases in infants and among elderly people (1.6 per 100000 population). In 2016, 375 cases were reported in France and in 2014 USA reported 675 confirmed cases. As reported by the Foodborne Diseases Active Surveillance Network in the USA, Listeriosis is a sporadic illness (Angel *et al*. 2019).

In Texas, listeriosis cases (n = 10) were confirmed from machine cut diced celery which was served in five different hospitals among the patients over 55 of age with underlying health issues (Gaul *et al*. 2013). Five patients died with listeriosis and all the ten patients with immuno-compromise conditions were under the treatment of corticosteroid which might have increased their susceptibility to invasive listeriosis. The outbreak strain could be isolated from the processing facility and from many bags of diced celery recovered from the manufacturing facility.

A multi-state outbreak was reported in USA in 2011 that occurred through eating cantaloupe. A total of 147 persons were infected causing 33 deaths and one miscarriage. As many as 99% of the patients were hospitalized mostly people above 60 years of age, and seven cases were associated with pregnancy. Five subtypes of *L. monocytogenes* were identified by PFGE typing. FDA confirmed that the strains 1/2a and 1/2b were acquired from the environment and food products (Robert *et al*. 2017). An outbreak occurred in 2014 in Illinois and Michigan by eating mung bean sprouts, in which five people became ill; all were hospitalized and two of them died. FDA confirmed *L. monocytogenes* presence in sprouts and irrigation water samples which were collected during a routine inspection. WGS revealed that all the isolates collected from food, environment and the patients were highly related. *Listeria monocytogenes* was still found in the subsequent inspection in that production environment (Robert *et al*. 2017).

In July 2014 in California, a packing company recalled various stone fruits such as whole peaches, nectarines, plums and pluots due to detection of *Listeria*. Four exact Pulsotypes were detected from patients by PFGE typing from the stone fruit samples (Robert *et al*. 2017). An outbreak occurred during 2014-2015 in California from Caramel apples affecting 35 people, of which 34 were hospitalized, 11 were pregnancy related, one resulting in fetal loss and seven died. Three invasive illnesses (meningitis) were reported. FDA isolated *L. monocytogenes* from the apple packing facility and also from the caramel apples. WGS revealed that the isolates were highly similar to those isolated from the patients (Robert *et al*. 2017). An outbreak of listeriosis was reported in March 2016 which was traceable to consumption of frozen vegetables produced by CRF Frozen Foods of Pasco, Washington. It was found that organic and frozen vegetables were the source of infection. The *Listeria* isolate from the frozen peas was found to be closely related with one isolate from an ill person based on WGS.

Multistate outbreak of listeriosis associated with raw milk produced by miller's organic farm in Pennsylvania was reported in 2016. The source of the illness was identified in 2016 only when the US Food and Drug Administration identified that the isolate from raw milk was closely related with *Listeria* isolates from the patients using whole genome sequencing (Anon 2016a). CDC and FDA investigated a multistate outbreak of listeriosis in 2016 linked with packaged salads produced in springfield at the Dole Processing Facility. Nineteen people were infected in the outbreak from nine states; all were hospitalized, one person died and one illness was related with pregnancy. Whole genome sequencing (WGS) showed that the isolates were closely related and the ill people in Canada were also infected with the same strain (Anon 2016b).

In 2017, eight people were infected in an outbreak reported from four states of US linked with soft raw milk cheese made by Vulto Creamery of Walton, New York. All were hospitalized, one illness was found in a newborn and two people died (Anon 2017). CDC reported a multistate outbreak of listeriosis linked with pork products in 2018 produced by Long Phung Food Products. The outbreak was over as of January 29, 2019 with four hospitalization cases (Anon 2018a). Eight people were infected with a *Listeria* outbreak reported in 2019 from five states of US which occurred due to eating of Hard-boiled Eggs from Almark Foods of Gainesville and Georgia. Five people were hospitalized and one death was reported (Anon 2019a).

Another Listeriosis outbreak was reported from 13 states in 2019 (n = 24). In all, 22 patients were hospitalized and two of them died. All people ranged in age from 35 to 92 years and 63 % percent of them were female. There was evidence of infections in several Canadian provinces connected with cooked diced chicken. WGS revealed that the type of strain which made people sick in Canada was similar with the strain in the United States, although the source of the infection was not identified (Anon 2019b).

In a recent report dated April 2020, FDA and CDC investigated a multistate outbreak of *L. monocytogenes* contamination

through enoki mushrooms imported from Korea. The total cases of illness were 36 with 4 deaths (Anon 2020). The overview of Listeriosis outbreaks recorded by CDC is shown in Table 2.

Table 2: Overview of Listeriosis Outbreaks by Contaminated Foods Identified by CDC in last 10 Years

Source of Samples	Year of Outbreak	References
Cantaloupes	2011	Robert *et al*. (2017)
Ricotta Salata Cheese	2012	Lomonaco *et al*. (2015)
Cheese	2013	Anon (2013)
Dairy Products	2014	Anon (2014a)
Cheese	2014	Anon (2014b)
Bean Sprouts	2014	Robert *et al*. (2017)
Caramel Apples	2014	Robert *et al*. (2017)
Ice Cream	2015	Anon (2015a)
Soft Cheeses	2015	Anon (2015b)
Raw Milk	2016	Anon (2016a)
Packaged Salads	2016	Anon (2016b)
Frozen Vegetables	2016	Anon (2016c)
Vulto Creamery Soft Raw Milk Cheese	2017	Anon (2017)
Prok Products	2018	Anon (2018a)
Deli Ham	2018	Anon (2018b)
Hard- Boiled Eggs	2019	Anon (2019a)
Unknown	2019	Anon (2019b)
Deli- Sliced Meats and Cheeses	2019	Anon (2019c)
Enoki Mushrooms	2020	Anon (2020)

LISTERIOSIS IN ANIMALS

Listeria monocytogenes can infect animal species such as mammals, birds, fish and crustaceans. In ruminants, listeriosis cases occur mostly clinically, while birds may suffer from sub-clinical infections and pigs rarely develop listeriosis. Most infections are sub-clinical, but sporadic. Ruminants may transmit the infection to humans through contaminated animal products. Direct transmission may occur rarely, especially during calving or lambing from infected animals (Wesley 2007). *L. monocytogenes* was first isolated in a laboratory animal breeding unit from an epidemic disease of rabbits and guinea-pigs (Murray *et al*. 1926). It was recorded that more than 40 species of wild and domesticated animals may be infected from this disease (Seeliger 1961). In the United Kingdom, listeriosis is most common in sheep and has a major veterinary importance in cattle, sheep and goats (Anon 1992). A number of conditions like uterine infections are associated with Listeric encephalitis (Wilesmith and Gitter 1986). Listeric encephalitis is a neurological disease of sheep described in New Zealand for the first time, locally known as 'circling disease' (Gill 1931). The organism, *L. monocytogenes*, was isolated from the lesions (Gill 1933) and listeric encephalitis of sheep, cattle and goats (Vandegraff *et al*. 1981). The clinical symptoms of the infection are due to the effect of the lesions in the brain stem (Rebhun and deLahunta 1982) with some common symptoms such as dullness, walking in circle and turning of the head to one side, drooping of the eyelid and ear due to unilateral paralysis of facial nerve. The animals also salivate because of partial pharyngeal paralysis. In case of sheep and goats, leaning and then death within 2 or 3 days, but it may take longer in case of cattles. Rare cases of listeric myelitis resulting in limb paralysis have been reported in sheep (Seaman *et al.* 1990). Listeric abortion occurs frequently in ruminants and many other species of domesticated animals caused by *L. monocytogenes* (Kennedy and Miller 1992). In the United Kingdom, listeric abortion was most commonly found in sheep (Anon 1992) which is usually sporadic (Kennedy and Miller 1992).

The first listeric abortion case in domesticated animal from India was reported from the uterine pus of ewe, from which *L. monocytogenes* was isolated (Dhanda *et al*. 1959). In Jammu and Kashmir, 111 abortion cases were recorded in sheep out of 800 lambings during 1977–78 and a serotype 4b strain of *L. monocytogenes* was isolated from one of 22 samples of stomach content of aborted foetuses (Vishwanathan and Uppal 1981). Investigation on migratory flocks revealed that *L. monocytogenes* and *L. ivanovii* could be isolated from vaginal swabs of sheep and goats with a history of abortions (Sharma *et al*. 1996). In Himachal Pradesh also, isolation of *L. monocytogenes* and *L. ivanovii* was reported from ewes (Nigam *et al*. 1999). *Listeria monocytogenes* was also isolated from aborted foetuses of a cow and buffalo (Dutta and Malik 1981). Massive occasional outbreaks of septicaemia were recorded in pregnant ewes (Low and Renton 1985). Septicaemia may also occur in the neonate as an extension of intrauterine infection which is relatively uncommon. Stored fodder and the environment are the main source of contamination of animal listeriosis. *Listeria monocytogenes* lives in soil, water and decaying vegetables and can contaminate animal feed mainly by silage (Wiedmann *et al*. 1997b). After oral ingestion, the bacteria enter into intestinal mucosa in case of septicaemic

listeriosis. In rhombencephalitis, i.e. inflammatory disease of hind brain (brainstem and cerebellum), *L. monocytogenes* invades the brain stem through cranial nerves usually with an incubation period of 2–3 weeks (Roberts and Wiedemann 2003).

Listeric mastitis results in culling of the infected animals from a herd. Recovery of the organism was reported from milk and milk products (Bhilegaonkar *et al*. 1997). *Listeria monocytogenes* and *L. ivanovii* were isolated from milk and faecal samples of mastitic cattle and buffaloes and also from endometritis cases in buffaloes (Shah and Dholakia 1983). Serotypes 1/2c and 4b of *L. monocytogenes* were isolated from the endometrium of infertile cows (Srivastava *et al*. 1985). *Listeria ivanovii* and *L. monocytogenes* were isolated from sheep and goats with endometritis from Himachal Pradesh (Mahajan and Katoch 1997). Incidence of *L. monocytogenes* was reported from 4.4% buffaloes with a previous record of reproductive disorders (Shakuntala *et al*. 2006). An outbreak was reported in a flock of 700 broiler chickens with neurological signs with a mortality rate of 40% (Vijayakrishna *et al*. 2000). *Listeria monocytogenes* has been recognised as one of the agents associated with subclinical mastitis; therefore, to reduce disease incidence and to avoid *L. monocytogenes* contamination in animals, creation of awareness among livestock farmers is important for its effective control. Animal production units via faecal contamination of food products are the main reservoir for *L. monocytogenes* and these are also the source for human infection (Esteban *et al*. 2009).

CONCLUSION

Listeria monocytogenes has been identified in human, animals and foods worldwide. Genital listeriosis is the most common clinical form in humans reported so far. In animals, spontaneous abortions, subclinical mastitis, meningo-encephalitis and endometritis are commonly reported. There is no reference laboratory for listeriosis in India, though the occurrence of listeriosis in man and animals has been reported from time to time.

To minimize the risk of foodborne Listeriosis in vulnerable populations, the types of food materials involved in the outbreaks should be identified. To ensure food safety at each stage of food handling, effective strategies may be implemented by integrating principles such as hazard analysis and critical control points (HACCP). Recent studies have indicated that improving control measures in some regions can decrease the prevalence of *L. monocytogenes* in food products. However, the percentage of illness particularly of invasive disease has remained stable, in some cases or has increased over a period. The possible reasons for increased reports of cases may be due to consumption of non-traditional food.

Listeria monocytogenes has been difficult to enumerate in the past due to limitations in laboratory detection and also its long incubation period, in addition to its ability to form biofilms, grow in refrigerated temperatures, and its resistance to disinfectants. Diagnostic advances used for early detection may be a factor correlated with the changing epidemiology in the recent years. Though PFGE is often used as a primary screening tool, the development of reliable sequencing technology has allowed identification of outbreaks more rapidly. In many countries, listeriosis is not a reportable disease, which makes it unrecorded during routine data collection (Desai *et al*. 2019).

Listeria monocytogenes strains can persist for years or decades in food processing plants is the primary source of post-processing contamination during manufacturing of food products and in retail or food service settings. The persistence may be due to the constancy and growth of some strains in niches within the food environment such as cracks and crevices of surfaces, seals and gaskets which are difficult to clean. Research on strain persistence has found the role of biofilm formation and physiological tolerance to sanitation or processing obstacles. Resistance to disinfectants which is used to sanitize food environments, equipment and utensils has been viewed as a possible mechanism for persistence. Recent studies have concluded that it is virtually difficult to permanently eradicate *L. monocytogenes* from food environments because of its ubiquitous nature. Therefore, eradication and suppression of the organism must be actively managed by adequate hygienic practices of a food premise and equipment, by effective cleaning, sanitation procedures and personnel practices (Robert *et al*. 2017).

Over the last decade many outbreaks have been linked with foods as an agent for *Listeria* transmission. To keep track of the food borne infections including listerosis, a national food safety code should be applied covering all particulars of Indian food safety under a united system. Creation of awareness among consumers is required to keep morbidity and mortality as low as possible. The epidemiological studies of listeriosis would help to understand the sources of infection, path of transmission and better management of the infection.

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